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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference P06031PC00	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/SE 03/01077	International filing date *(day/month/year)* 23.06.2003	Priority date *(day/month/year)* 21.06.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/10		
Applicant SINOGENOMAX COMPANY LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand 12.01.2004	Date of completion of this report 30.09.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer van Heusden, M Telephone No. +49 89 2399-8145

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/SE 03/01077

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-14 as originally filed

Claims, Numbers

1-20 received on 16.09.2004 with letter of 14.09.2004

Drawings, Sheets

1/8-8/8 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 16-18, 20

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☒ the claims, or said claims Nos. 16-18, 20 are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11, 13-15, 19
	No:	Claims	12
Inventive step (IS)	Yes:	Claims	2, 19
	No:	Claims	1, 3-15
Industrial applicability (IA)	Yes:	Claims	1-15, 19
	No:	Claims	

2. Citations and explanations

   see separate sheet

Additional remarks to section III:

1. The omission from **claim 1** that the dsRNA encoding the dsDNA should be randomized does not seem to be supported by the description as filed: the passage indicated by the applicant on p. 6 relates to a specific example (Renilla luciferase) which cannot be generalized. Furthermore the indicated paragraph finishes by referring to the key finding of the present invention which makes it possible to construct a fully randomized siRNA library (l. 10-12). It appears that the entire application relates to the provision of a library of randomized dsRNA molecules. Thus it appears that **claim 1 (and 2)** should refer to randomized dsRNA-encoding sequence. Examination has been performed on said claims assuming they would relate to randomized dsRNA-encoding sequences.

2. In **claim 6** the reference to a poly-U overhang in general does not seem to be disclosed in the application as filed, which only refers to a 3' poly-U overhang (p. 3). Examination has been performed on said claim assuming it would relate to 3' poly-U overhang.

3. It seems that in **claims 14 and 15** the use of 'the RNA library according to claim 12' for the indicated method of screening is not disclosed in the application as filed. Examination has been performed on said claims assuming they would relate only to the DNA-library according to claims 1-10.

4. The subject matter of **claims 16 and 17** is not disclosed in a direct and unambiguous manner in the first paragraph on p. 1 of the application. These claims have not been examined.

5. **Claim 18** relates to the use of a DNA molecule as defined. It seems that the application only discloses DNA vectors comprising the sequences as indicated. Furthermore no basis can be found for the specific molecule as defined in the claim: the description only discloses said molecule as part of a larger molecule including specific H1 promoter sequences (the termination sequences are accommodated into the promoters by mutation! and not attached to the promoter sequences) and not as an isolated molecule of only $AAAAA(N)_nTTTTT$. Furthermore no basis can be found for the specific lengths of 19, 20 or 21 nucleotides in combination with the general formula $AAAAA(N)_nTTTTT$. Thus claim 18 has not been examined.

6. The applicant has indicated p. 6 (l. 7-9) and Figure 2 as a basis for the subject matter of **claim 20**. Said passage relates to a specific example of Renilla luciferase siRNA defined by a specific sequence as disclosed in Figure 2, flanked by two mutated RNA polymerase III promoters, each embedding one transcription terminator sequence for the other promoter. Claim 20, in contrast, refers to any siRNA-encoding region, which seems to be a generalization which is not disclosed in the application as filed. Thus claim 20 has not been examined.

Additional remarks to section V:

1. Citations

1.1 The documents mentioned in this report are numbered as in the International Search Report (ISR), i.e. D1 corresponds to the first document of the ISR etc.

2. Novelty (Article 33(2) PCT)

2.1 The present application relates to a DNA library of dsDNA wherein each dsDNA comprises a stretch wherein both strands encode a promoter, a dsRNA-encoding sequence of 10-30 base pairs and a transcription termination sequence, wherein each of said promoters has been mutated to include the sequence complementary to the termination sequence of the other strand. It further relates to a kit comprising said library and to an RNA-library obtained from said DNA library. It also relates to a method of screening for dsRNA with biological functions or for novel genes, using said library.
It further relates to the use of a DNA molecule comprising the DNA sequence AAAAA(N)nTTTTT in the production of dsRNA molecules, and to an H1-polymerase III-promoter mutated to have AAAAA at the end of the promoter.

2.2 The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject matter of claim 12 does not seem to be novel: the RNA-library according to claim 12 is defined as a product by process (obtained from the DNA-library of claim 1-10). The process feature in a product claim can only be relied on for establishing novelty over the prior art, where use of that process necessarily means that the product has a **particular characteristic** and the skilled person, following the teaching of the specification, would inevitably achieve that characteristic, **would be aware of that characteristic** and would discard any

products not having it. In the present case it is not clear how the RNA-library obtained from the DNA-library according to claim 1-10, could be discriminated from an RNA-library made e.g. by chemical synthesis, and having e.g. 4 or more positions randomized. Therefore the subject matter of claim 12 cannot be considered novel.

3. Inventive step (Article 33(3) PCT)

3.1 The present application does not seem to satisfy the criterion set forth in Article 33(3) PCT because the subject matter of claims 1 and 3-15 does not appear to involve an inventive step in view of document D1, which discloses an expression vector comprising a sequence encoding a sense and antisense sequence of 19 nucleotides corresponding to a gene of interest, each under the control of a U6 promoter. D1 suggests on p. 499, left hand column, second paragraph, the production of randomized siRNA libraries and their use for genetic screens. D1 further suggests the use of opposing promoters and refers to the use of opposing T7 promoters in D11. D1 further states that in preliminary experiments opposing U6 promoters were developed.

The subject matter of the present claims differs from the disclosure in D1 in that a DNA library is provided, rather than a single vector, and in that said library consists of dsDNA wherein both strands encode a promoter, a dsRNA-encoding sequence of 10-30 base pairs and a transcription termination sequence, and wherein each of said promoters has been mutated to include the sequence complementary to the termination sequence of the other strand.

Therefore the objective problem to be solved by the present application can be seen as the provision of a further dsDNA library encoding dsRNA molecules.
D1 does not suggest the use of termination sequences, even less so to mutate the promoter sequence such as to incorporate the termination sequence immediately preceding the transcription start site.
As indicated in the application (p. 5, l. 34) it could not be predicted how the insertion of an AAAAA stretch would affect the activity of the promoter (transcription starting and rate of transcription). The applicant has shown that the mutation of an H1 RNA polymerase III promoter such as to incorporate the AAAAA sequence at the end of the promoter results in proper and effective transcription. Therefore an inventive step can be recognized for said mutated H1 RNA polymerase III promoter and its applications in a DNA-library. Thus the

subject matter of claim 2, insofar as it relates to the H1 RNA-polymerase III-promoter and claim 19 is considered inventive.
With respect to other promoters, it can equally not be predicted how the function of any promoter will be affected by mutation of the end of the promoter to accommodate the complementary sequence of any termination sequence.
Therefore it appears that the subject matter of claim 1 is not enabled over the full scope of the claim (any promoter and any termination sequence). The same applies to the subject matter of claims 3-15.

4. Industrial applicability (Article 33(4) PCT)

The subject matter of claims 1-15 and 19 appears to be industrially applicable.

CLAIMS

1. A DNA-library for production of a library of double stranded RNA-molecules (dsRNA) of a predefined length, the library consisting of double stranded DNA-molecules (dsDNA) where each dsDNA comprise a stretch wherein both strands contiguously encode a promoter, a dsRNA-encoding sequence of 10-30 base pairs encoding the dsRNA to be produced and a transcription termination sequence, wherein each of said promoters has been mutated to include the sequence complementary to the termination sequence of the other strand.

2. A DNA-library according to claim 1, wherein said promoters are H1-promoters or U6-promoters that have been mutated so as to incorporate an AAAAA-stretch at the end of the promoter, immediately next to the transcription starting site.

3. A DNA-library according to claim 1 or 2, wherein said dsRNA-encoding sequence is randomized in between 4 and all positions.

4. A DNA-library according to any of claims 1-3, wherein the produced dsRNA contains a single stranded region at one end.

5. A DNA-library according to any of claims 1-3, wherein the produced dsRNA contains single stranded regions at both ends.

6. A DNA-library according to claim 4 or 5, wherein at least one of the single stranded regions of the dsRNA is a poly-U overhang.

7. A DNA-library according to claims 4 or 5, wherein at least one of the single stranded regions of the dsRNA is a UU overhang.

8. A DNA-library according to any of claims 1-7, wherein it is constructed in a plasmid vector.

9. A DNA-library according to any of claims 1-7, wherein it is constructed in a viral vector.

10. A DNA-library according to any of claims 1-9, wherein the randomness of the library was modified by selection of the random DNA oligonucleotides, before cloning the said random DNA oligonucleotides into the vectors, through hybridization to a total RNA preparation or total mRNA preparation from a source, whereby only the oligonucleotides

hybridized to the source RNA (or mRNA) are subsequently cloned into the vector, and wherein the source can be a cell, a cell line, a tissue, or a organism.

11. A kit containing the DNA-library according to any of claims 1-10.

12. An RNA-library obtained from the DNA-library according to any of claims 1-10.

13. A method of using the DNA-libraries of any of the claims 1-10, wherein the library is transiently or permanently introduced into cells as a mixture.

14. A method of screening for double stranded RNA with biological functions comprising the use of the DNA-library according to any of claims 1-10 or the RNA-library according to claim 12.

15. A method of screening for novel genes comprising the use of the DNA-library according to claims 1-10 or the RNA-library according to claim 12.

16. An individual DNA-member of the DNA-library according to any of the claims 1-10.

17. An individual RNA-member of the RNA-library according to claim 12.

18. Use of a DNA-molecule comprising the DNA-sequence $AAAAA(N)_nTTTTT$, wherein $(N)_n$ is a randomized region of 19, 20 or 21 nucleotides, in the production of dsRNA-molecules.

19. An H1 RNA-polymerase III-promoter mutated to have an AAAAA-stretch at the end of the promoter immediately ahead of the transcription starting site.

20. A plasmid with two mutated RNA polymerase III promoters, each embedding one transcription termination sequence for the other promoter, and a siRNA-encoding region between the promoters.